Screening for Phenylketonuria

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Key Words

Newborn screening · Filter card · Dry blood spot · Phenylalanine · Tandem mass spectrometry

Abstract

Newborn screening for phenylketonuria (PKU) started with Robert Guthrie (1916–1995) who developed the bacterial inhibition test for the semiquantitative analysis of phenylalanine, which was the first test suitable for high throughput analysis. In addition, he introduced the 'Guthrie filter card' as a transport medium for dried blood which is still used today. Realizing the potential of his approach for early diagnosis and the fact that a low-phenylalanine diet prevented the neurological sequelae of untreated PKU, Robert Guthrie became the first and utmost advocate of newborn screening for PKU. Following the first PKU newborn screening program in Buffalo, N.Y., USA, in the 1960s, additional newborn screening programs were initiated around the world in the 1960s and 1970s. Newborn screening has since been recognized as an important public health measure, and most countries have ongoing newborn screening programs for PKU and other inborn errors of metabolism. Since the first programs, it has been recognized that early diagnosis of PKU and subsequent initiation of a low phenylalanine diet results in normal neurological outcomes – in contrast to the severe mental retardation in untreated PKU. Today's newborn

screening laboratories use photometric assays or tandem mass spectrometry for analysis of phenylalanine rather than the bacterial inhibition test. This has led to an increased number of cases with hyperphenylalaninemia that often do not require dietary treatment. Any elevated phenylalanine level in a neonate needs to be followed by a second specimen for repeat analysis of phenylalanine and tyrosine. Confirmation of elevated phenylalanine levels with low to normal tyrosine levels requires analysis of urine pterines and dihydropterine reductase activity in red cells to rule out an inborn error of biopterin metabolism. In any neonate whose blood phenylalanine levels exceed 6 mg/dl, a tetrahydrobiopterin loading test should be performed and dietary therapy should be initiated. The level at which dietary therapy is started may be different between the USA, UK and continental Europe.

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History of Newborn Screening for Phenylketonuria

The seminal work of the microbiologist and physician Robert Guthrie (1916–1995) in the 1960s laid the groundwork for modern newborn screening as we know it today. Due to the mental retardation of his second child, Dr. Guthrie became involved in the New York State Association for Retarded Children, where he became vice-presi-

dent of the local Buffalo Chapter. Dr. Robert Warner, who was director of the Children's Rehabilitation Program at the Children's Hospital in Buffalo, N.Y., USA, explained phenylketonuria (PKU), a disorder of phenylalanine metabolism and recently identified cause of mental retardation, to Dr. Guthrie. He also mentioned the fact that affected children tend to get better on a phenylalanine-restricted diet and that one of the major problems in administering the diet was the monitoring of phenylalanine blood levels due to the lack of suitable analytical techniques [1–4]. Using his expertise in microbiology, Dr. Guthrie developed an intriguing yet simple test – the bacterial inhibition test – to screen large numbers of newborn infants for the presence of elevated levels of blood phenylalanine. Details of this test are described below [1, 2]. However, this test could not have been readily applied if it were not for the development of the filter paper – ‘Guthrie card’ – which was used as a transport medium for the neonatal blood. When asked years later as to his contribution, Dr. Guthrie answered that his most important contribution with the largest impact was the development of the filter paper that is still in use today (fig. 1) [1, 2, 5].

Dr. Guthrie was instrumental in bringing the bacterial inhibition test to widespread use through the implementation of regional and national screening programs in the 1960s and 1970s. Dr. Guthrie declined to patent or accept royalties for his test, which made the test affordable for all hospitals. The first neonatal screening program for PKU was initiated in the state of New York in the early 1960s [3].

Newborn Screening – Principles and General Comments

Newborn screening is defined as the screening of all infants typically at day 3–5 of life within a defined region for the presence of inborn errors of metabolism, endocrinopathies, cystic fibrosis, hemoglobinopathies and others depending on the particular requirements [6, 7]. Newborn screening has been recognized as an important public health measure with direct benefits for the affected individuals and their families and indirect benefits for the society as a whole.

Disorders that are screened for have to follow criteria that were developed in 1968 by Wilson and Jungner as part of a WHO initiative on principles and practice of screening for disease [8]. Wilson and Jungner stated: ‘The central idea of early disease detection and treatment is

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Fig. 1. The blood of a neonate is collected onto a Guthrie card. USAF photographic archives.

essentially simple. However, the path to its successful achievement (on the one hand, bringing to treatment those with previously undetected disease and, on the other, avoiding harming those not in need of treatment) is far from simple though sometimes it may appear deceptively easy’. These basic principles are still valid today, although they have been adapted to the requirements of modern newborn screening [9]. Ideally, disorders that are screened for should be reasonably frequent and they should constitute a significant public health problem. The natural disease course should be understood, and there should be a form of treatment or intervention that alters the natural disease course to the benefit of the affected patient [8–10]. It is obvious that a sensitive and specific screening test, which can be readily applied in dry blood spots in a large number of neonates, is a prerequisite of newborn screening for any disorder of interest. In many ways, PKU has led the way and was the first disorder to fulfill all criteria, maybe with exception of those countries where the birth incidence of PKU is low, such as Finland and Japan [11, 12].

Newborn screening identifies neonates that are at risk of having a particular disorder but no definitive diagnosis is provided. For that reason, confirmatory diagnosis, ideally following a diagnostic algorithm, has to be done in any suspicious case (fig. 2). Ultimately, a number of cases may be found to be false positive, causing increased parental stress and perturbed parental bonding and overreaction in the future [13]. When newborn screening for PKU was introduced, some infants with false-positive screening results were started on a phenylalanine-restricted diet, but apparently did not suffer from any adverse outcomes [14].

Fig. 2. Newborn screening algorithm for PKU. Adapted from the ACMG ACT sheet for PKU. * USA/UK; Germany >10 mg/dl.

Although the number of disorders that are screened for vary from country to country, PKU is included in most if not all screening programs around the world [6, 7].

Analytical Techniques for Screening

There are several analytical techniques that can be used for quantitative and semi-quantitative analysis of phenylalanine levels from dry blood spots [15]. With the introduction of expanded newborn screening, most screening laboratories now use tandem mass spectrometry (MS/MS) for the analysis of amino acids including phenylalanine and acylcarnitine species [16].

Bacterial Inhibition Test

Bacterial growth on an agar plate is inhibited through the action of a particular chemical ('inhibitor') on a small disc in the middle of the plate. Any structurally related compound, e.g. amino acid (phenylalanine) or metabolite, will compete with the inhibitor and initiate bacterial growth. The growth zone around the punch will be proportionate to the amount of phenylalanine, e.g. blood concentration that is brought onto the plate with a dry blood punch. The size of bacterial growth zones from standard blood samples can be compared with those from individual neonatal samples and the blood phenylalanine concentration deduced accordingly in a semi-quantitative manner [2, 3]. Antibiotics given to the mothers and/or infants may interfere with the results of any bacterial inhibition test as bacterial growth may have been inhibited. The bacterial inhibition test was also used

for the detection of histidinemia, maple syrup urine disease and other enzyme deficiencies [3, 17].

Tandem Mass Spectrometry

MS/MS was introduced to newborn screening laboratories during the late 1990s [18–20]. The main advantage of this technique is the simultaneous, fully automated analysis of different analytes such as amino acids including phenylalanine and acylcarnitine species [16]. The diagnostic sensitivity of MS/MS for hyperphenylalaninemia (HPA)/PKU is superior compared to other analytical techniques. The measurement of the phenylalanine to tyrosine ratio may help in differentiating between false positives and cases of HPA/PKU [15].

Newborn Screening for PKU

A detailed algorithm for follow-up of elevated phenylalanine levels in newborn screening for PKU is depicted in figure 2. This algorithm follows the ACT and FACT sheets developed by the American College of Medicine [21]. Details can be found at www.acmg.net/resources/policies.

Any elevated phenylalanine level found in a newborn screening sample should be followed by a second sample, either a dry blood filter card or a whole blood sample. The latter may have the advantage that it can be used for accurate analysis of all amino acids and is preferred when the first phenylalanine is significantly elevated (e.g. above 3 mg/dl) [22]. Phenylalanine may be elevated in preterm and/or critically ill infants due to catabolism and/or total parental nutrition (TPN). Under these circumstances, all amino acids should be analyzed in whole blood preferably in a fasted state or following a brief period without TPN. In any preterm infant which is born before 33 weeks of gestation, newborn screening is typically repeated after 14 days [6]. However, these recommendations may vary from program to program.

Antibiotics given to the mother just prior to delivery or the infants during the first days of life do not affect the screening result for PKU unless the bacterial inhibition test is used [3].

Elevated phenylalanine levels in neonates may not only be secondary to PKU or HPA but may also be observed in the less frequent disorders of bipterin metabolism [23]. There are five different disorders that affect tetrahydrobiopterin (BH₄) synthesis or recycling including deficiencies of GTP cyclohydrolase I, 6-pyruvoyl tetrahydropterin synthase, sepiapterin reductase, dihydropteridine reductase

(DHPR) and pterin-4 α -carbinolamine dehydratase [23]. Consequently, urine pterines and DHPR activity in red cells have to be analyzed in any neonate with confirmed elevated phenylalanine levels to rule out disorders of bipterin metabolism. When such a disorder is diagnosed, therapy with BH₄ and neurotransmitter precursor has to be initiated promptly [23]. Phenylalanine levels are not elevated in patients with sepiapterin reductase deficiency and may only be transiently elevated in patients with pterin-4 α -carbinolamine dehydratase deficiency [24–26].

Screening for PKU in At-Risk Populations

Besides newborn screening for PKU, several studies have demonstrated a relatively high prevalence of PKU in mentally retarded, previously unscreened children and adults [27, 28]. For example, the prevalence of PKU in an unselected population of mentally retarded adults in Spain was 0.3% (3/944) [27], whereas a similar study in Iran identified a prevalence of 2.1% (104/4,963) [28]. These differences may not be readily explained but may be due to recruitment bias, different analytical techniques used and variable frequencies of PKU in the respective populations [29].

Cost-Benefit Assessment of Newborn Screening for PKU

Newborn screening for PKU changed the prevalence of mental retardation in previously untreated patients with PKU from 95% to less than 1% [25]. Initial cost-benefit assessments concluded that newborn screening for PKU is cost beneficial as mental retardation and the subsequent institutionalization and the related costs are essentially avoided. Normally-developed patients with PKU instead can join the work force and contribute to social taxes [30, 31]. Newer assessments demonstrated that newborn screening for PKU may be not as cost effective as previously thought as the overall structure of public health has changed considerably over the last 30 years [32]. Dietary treatment is now recommended for life and is relatively expensive, whereas children with mental retardation typically live at home [32–37]. In addition, children born to mothers with PKU are at risk of birth defects if the mother is not well controlled before and during early pregnancy (maternal PKU) [38]. In contrast, newborn screening for PKU in Finland may not be cost beneficial due to the low incidence of PKU [11].

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